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Root Exudates of Fifteen Common Weed Species: Phytochemical Screening and Allelopathic Effects on *T. aestivum* L.

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Abstract: Through allelopathic interactions, plants may either suppress competing species or promote those that may help them better adapt to their environment. The purpose of this research was to determine how the root exudates of 15 common weeds affected the germination of wheat (*Triticum aestivum* L.). Every other day, 15 seeds were dispersed over Petri dishes (with filter paper) and pots (with garden soil) and treated with 1 mL and 5 mL of aqueous root exudates, respectively. Distilled water had the highest germination rate at 86.33% and the lowest at 64.00% ($p = 0.001$) for *Commelina benghalensis* in the Petri dish containing root exudates. The seed germination percentage of the pot condition was the lowest, at 68.45% ($p = 0.004$), for *Solanum nigrum*, while the control was 87.23%. Similarly, the lowest shoot length, 12.01 cm ($p = 0.0025$) in *Mikania micrantha,* and the lowest root length of 2.17 cm ($p = 0.0048$) in *Leucas aspera,* were recorded, whereas the control was 19.13 cm and 3.46 cm, respectively, in Petri dishes. In addition, the lowest shoot and root growth were 9.72 cm ($p = 0.0004$) in *Mikania micrantha* and 4.34 cm ($p = 0.0019$) in *Spilanthes acmella*, while the control was at 20.13 cm and 6.42 cm, respectively, for pot culture. Furthermore, in seedlings, biomass studies of treated *T. aestivum* showed elevated malonaldehyde (MDA) levels in both Petri dishes and pot cultures. However, chlorophyll a and b levels, as well as those of the antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD), are lower than in the control group. Both enzymes were elevated in pot cultures compared to those grown in Petri plates. The dry weight of *T. aestivum* seedlings cultivated in pots and Petri dishes demonstrated its considerable allelopathic influence. This research found that the root exudates of all the weeds studied could have the capacity to impede wheat seed germination as well as the development of wheat seedlings. These inhibitory effects were higher in Petri dishes than in pot cultures.

Keywords: weeds; root exudates; allelochemicals; phytotoxicity; seed germination; seedling growth

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1. Introduction

Weeds in agricultural fields compete with crop plants for light, moisture, and essential nutrients, lowering crop quality and yield while raising production costs [1]. Allelochemicals produced by weed species are supposed to be more toxic because weeds typically grow under stress conditions [2,3]. Allelochemicals can be used to achieve long-term weed control using allelochemicals as natural herbicides or allelopathic interactions [4]. Numerous new compounds, including 5-chloro-2-nitromethylbenzo[d]oxazoles and 4-2-nitromethylbenzoxazoles, may have herbicidal action [5] and were therefore exploited to create bioherbicides. By employing them as cover crops, surface mulch, residue integration, intercropping, rotation, and crop extract with a decreased dosage of pesticides, several crop species possess the allelopathic potential that may be utilized to control weeds in field crops [6]. Thus, it is possible to look for allelopathic features in various crop varieties.

Common field crops including rice (*Oryza sativa* L.), wheat (*T. aestivum* L.), sunflower (*Helianthus annuus* L.), maize (*Zea mays* L.), canola (*Brassica napus* L.), sorghum (*Sorghum bicolor* L.), millet (*Pennisetum glaucum* (L.) R.Br.), and buckwheat (*Fagopyrum esculentum* Moench) possess a variety of allelochemicals that can be used to suppress weeds [4].

The indigenous cereal crops contain different allelochemicals that could suppress the population and growth of weeds [7]. Many weeds are increasingly gaining importance as weed control agents due to the presence of certain allelochemicals. These allelochemicals can inhibit the germination and growth of a variety of different weeds, including herbicide-resistant weeds [8]. Allelopathic effects can be either positive or negative, depending on the donor and recipient species, their growth stages, the rate at which allelochemicals are released into the environment, and the role soil microorganisms play in either cleaning up or increasing the phytotoxicity of released allelochemicals [9,10]. Allelopathy, as a purely natural mechanism, exerts its influence through root exudates, volatiles, foliage leachates released from intact plants, dead plant tissues, or decayed dried materials laid on or incorporated in the soil [11].

The application of herbicides and manual and mechanical removal is the most extensively researched control approach among the many weed management strategies that have been created throughout time for various systems. Due to their remarkable effectiveness, synthetic herbicides have traditionally been used to control weeds. However, since these chemicals are used indiscriminately, resistant weeds grow, severely affecting agriculture, human health, and the environment. The difficulties posed by the usage of pesticides make the development of alternative, environmentally friendly techniques essential [12,13].

Additionally, unlike the plant extract approach, which involves artificially killing cells and their organelles in order to extract the plant tissues or cells using water or organic solvents, the natural allelopathy process functions without damaging the donor plants unless the allelochemicals are autotoxic [14,15]. However, the search for naturally occurring phytotoxic chemicals that may be used to substitute synthetic pesticides in agriculture cannot be dismissed. In order to help the pesticide and permeate the plant, the root exudate may change the waxy coating or lessen the surface tension of the leaf. Factors that could accelerate plant development include hormones, nutrition, and secondary metabolites. Exudate may be used as a natural pesticide, either alone or in conjunction with synthetic pesticides, which might reduce environmental issues [15–18]. This study found that the effects of the aqueous root exudates of 15 weeds inhibited seed germination and seedling growth of *T. aestivum*. Moreover, it also reduced chlorophyll production in seedlings. Ex-vivo antioxidant enzymes such CAT and SOD, and MDA levels indicated the negative effects of root exudates.

2. Materials and Methods

2.1. Collection and Preparation of Root Exudate of Weeds

The aim was to determine the allelopathic effects of 15 weeds' root exudates on the seed germination ability and biomass production of wheat (*T. aestivum*) seedlings. The experiments were carried out three times in the laboratory of botany at the University of Chittagong, Bangladesh. A total of 15 alien invasive weed species were collected around the botanical garden at the University of Chittagong, listed in Table 1 with their natural status and verified by in-depth literature studies and a website (worldfloraonline.org accessed on 27 November 2022). Each weed species was dug out from the soil in the agricultural field, and the roots were then rinsed with tap water and then distilled water. After that, the plant roots were immediately moved to a conical flask filled with 400 mL of distilled water and maintained there for five hours so they could exude root fluid. Whatman No. 1 filter paper was used to filter this exudate. For the bioassay experiment, around one-fourth of the exudate was utilized. The remaining 100 mL was concentrated up to 25 mL using a water bath at 60 °C and kept in the refrigerator for biochemical analysis. The bioassay experiment employed the later exudates.

Table 1. Field status of weed species collected from the crop fields around the botanical garden of Chittagong University campus.

Weeds Species	Code	Family	Life Cycle	Growth form	Status of the Weeds In the Crop Fields
Centella asiatica (L.) Urb.	T1	*Apiaceae*	P	Herb	+++
Rotala indica (Willd.) Koehne	T2	*Lythraceae*	P	Climber	++
Solanum nigrum L.	T3	*Solanaceae*	P	Herb	++
Commelina benghalensis L.	T4	*Commelinaceae*	A/P	Creeping Herb	+++
Marsilea quadrifolia L.	T5	*Marsileaceae*	P	Herb	++
Ageratum conyzoides L.	T6	*Asteraceae*	A/P	Herb	++
Cynodon dactylon (L.) pers.	T7	*Poaceae*	P	Herb	+++
Spilanthes acmella L.	T8	*Asteraceae*	A/P	Herb	++
Heliotropium indicum L.	T9	*Boraginaceae*	A	Herb	+++
Leucas aspera (Willd.) Link	T10	*Lamiaceae*	A	Herb/Shrub	++
Phyllanthus niruri L.	T11	*Phyllanthaceae*	A	Herb	++
Sida acuta Burm.f	T12	*Malvaceae*	A/P	Shrub	+++
Mikania micrantha Kunth	T13	*Asteraceae*	P	Climbing	+++
Polygonum hydropiper L.	T14	*Polygonaceae*	A	Herb	++
Physalis heterophylla Nees	T15	*Solanaceae*	P	Herb	+

+: Low concentration, ++: Moderate concentration, +++: High concentration. A = Annual, B = Biennial, P = Perennial.

2.2. Seed Germination Assay

The wheat grain (*T. aestivum* L.) was used as a test plant for root exudate. The wheat seeds were bought at Chittagong's Hathazari neighborhood market. After being chosen for their consistent size, shape, color, and health, seeds were sterilized in 70% ethanol for 1–2 min to get rid of the chemicals. They were then rinsed five times with sterile distilled water. In 9-cm glass Petri dishes with Whatman No. 1 filter paper filters, 15 seeds were placed. They were then watered every other day for ten days with 1 mL of different aqueous weed root exudates. Garden soil pots of 5.5 cm × 5 cm were used for the soil experiment and the same number of seeds were planted in them while irrigating them with 5 mL of aqueous root exudates. There was wetness where the Petri dishes and other containers were placed, and the growth chamber was maintained at a constant temperature of 25 °C. The seed was deemed to have germinated when the radicle length exceeded 2 mm. Biochemical markers were assessed after 10 days, including the number of seeds that germinated, the size of the roots and shoots, and the weight of the seedlings.

Mean germination time (MGT) was calculated according to the following equation:

$$MGT = \Sigma\, Dn/\Sigma\, n,$$

where n is the number of seeds which germinated on day D, and D is the number of days counted from the beginning of germination.

Final germination percentage (FGP) was measured according to the formula:

$$FGP = (\text{No. of seeds germinated}/\text{No. of seeds sown}) \times 100.$$

2.3. Phytochemical Screening of Root Exudate

According to Cromwell [19], the following methods were used to conduct qualitative alkaloids tests of root exudates: For Dragendorff's reagent test, 1 mL of each sample solution was taken in a test tube and a few drops of Dragendorff's reagent (potassium bismuth iodide solution) were added. A reddish-brown precipitate was observed, indicating the presence of alkaloids. In the case of Meyer's reagent test, 1 mL of each sample solution and a few drops of Meyer's reagent (potassium mercuric chloride solution) were added. A cream-colored precipitate was formed, indicating the presence of alkaloids. For Wagner's reagent test, a few mL of each sample solution was added to Wagner's reagent (iodine in potassium iodide), resulting in the formation of a reddish-brown precipitate, indicating

the presence of alkaloids. In the Hager's reagent test, 1 mL of each sample was mixed with a few drops of Hager's reagent (picric acid). The yellow precipitate was formed by reacting positively with alkaloids. For the tannic acid test, a few mL of 10% tannic acid were added to 1 mL of each sample, and a buff-colored precipitate was formed, giving positive results for alkaloids. In the case of $FeCl_3$, a drop of this solution was added to each test sample. The formation of the yellow precipitate resulted in the samples reacting positively to alkaloids.

Moreover, for the test of carbohydrates, a few drops of the Molisch reagent (10 g α naphthalin in 100 mL of 95% alcohol) were added to 2–3 mL of the test sample. Then a few drops of added concentrated H_2SO_4 were mixed with it through the test tube wall. The formation of a purple-violet color ring at the junction indicated the presence of carbohydrates [20]. For flavonoids, a few drops of concentrated hydrochloric acid were added to a small amount of the root exudate of the plant material. The rapid development of the red color was taken as an indication of the presence of flavonoids. To test for phenolics, the exudate was mixed with 1% $FeCl_3$. The formation of blue, violet, purple, green, or red-brown stains indicated the presence of phenols. Finally, the test for proteins (Biuret test) was performed by adding 4% NaOH and a few drops of a 1% $CuSO_4$ solution to 3 mL of the exudate. The formation of a violet or pink color indicated the presence of proteins [21].

2.4. Estimation of Chlorophyll Content

Hiscox and Israelstan's [22] approach was used to calculate the chlorophyll content. During the first hour at 65 °C, 10 mL of dimethyl sulfoxide (DMSO) was absorbed into 100 mg of freshly picked, coarsely chopped leaves from each plant. After decanting, the DMSO evaporated and more DMSO was added to bring the total to 10 mL. Chlorophyll concentrations in DMSO-extracted samples were measured in a spectrophotometer at 645 and 663 nm, with DMSO acting as a blank. Arnon's [23] equation, revised by Hiscox and Israelstan [22], was used to calculate the total chlorophyll content.

2.5. Determination of CAT

The analysis of catalase (CAT) performance conformed to the recommendations made by Beers and Sizer [24]. A total of 0.5 mL of 75 mM hydrogen peroxide (H_2O_2), 0.05 mL of enzyme extract, 0.95 mL of ultrapure distilled water, and 1.5 mL of 100 mM potassium phosphate buffer were combined to make a reaction volume of 3.0 mL. No enzyme extract was present in a "blank" solution. The blank solution was heated in the spectrophotometer for around 4 to 5 min to achieve temperature equilibration. The absorbance at a wavelength of 240 nm was measured for 2 min using a spectrophotometer (UV-Vis 200, Shimadzu, Japan). The catalase enzyme activity required to degrade one mole of H_2O_2 was taken as one unit. Catalase activity was measured in micromoles per minute per gram of fresh plant (seedlings) tissue ($Mmin^{-1}$ g^{-1} FW).

$$\text{CAT } (\mu\text{mol min}^{-1}\text{ mL}^{-1}) = (240/\text{min}) \times \text{total volume} \times 1000/\ 43.6 \times \text{enzyme volume}.$$

To calculate the extinction coefficient, we measured the absorbance at 240 nm for 1 min.

2.6. Determination of SOD

Kakkar et al. and Fridovich's [25,26] techniques were used to measure superoxide dismutase (SOD). After weighing in at 1 g, the tissues were homogenized in cold KCl (0.15 mol/L) using an FSH-2A, YUEXIN YIQI China homogenizer for further study. The SOD was estimated using sodium pyruvate phosphate and phenazine methosulfate. The tissue combination was centrifuged at 4 °C for 60 min at 15,000 rpm. To 200 µL of 0.1 M EDTA (containing 0.0015% NaCN), 100 µL of the clear supernatant was added. Then, 2.95 mL of phosphate buffer (67 mM, pH 7.8) and 100 µL of 1.5 mM NBT were added. Absorbance at 560 nm was taken after adding the riboflavin and comparing the results to those obtained with deionized water. Absorbance was remeasured at 560 nm after the

tubes were exposed to uniformly illuminated incandescent light for 15 min. The proportion of inhibition was determined by contrasting the sample and control absorbances. The activity of SOD was measured in units per milligram of protein (U/mg protein), with one U denoting the removal of 50% of the superoxide anion that was produced.

2.7. Determination of LPO

The method for lipid peroxidation (LPO) was adjusted from that mentioned by Högberg et al. [27]. For the investigation, the tissues were weighed at 1 g and homogenized in cold KCl at 0.15 mol/L using an FSH-2A homogenizer from Yuexin Yuqi, China. The total volume of the solution was 2 mL after adding 0.3 M Tris-HCL buffer (pH 7.4) and 0.02 mM sodium pyrophosphate to the test tubes containing 0.2 mL of tissue homogenate. The reaction mixtures were heated to 37 °C in a water bath for 30 min of incubation. The reaction was stopped by adding 1 mL of 10% TCA, and the mixture was then incubated once more. Then, 1.5 mL TBA was added after a quick vortex and the reaction mixture was heated in a boiling water bath for 20 min. There were three runs of the trials. Following centrifugation, the color creation in all laboratory test tubes containing the reaction mixture was monitored at 532 nm. The results were then expressed as the amount of MDA per milligram of protein (nmol MDA/mg protein) that interacted with thiobarbituric acid.

2.8. Statistical Analysis

The data were analyzed using GraphPad Prism Data Editor for Windows, Version 8.4.3, using Dunnett's multiple comparison tests, including one-way and two-way ANOVA. All data were presented as mean, standard deviation (SD) and p-values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Germination Percentage

In terms of the germination rate, aqueous root exudates from weed species that were present in the Petri dishes suggested a significant degree of suppression. The physicochemical properties of the soil in a container are outlined in Table 2. There were significant differences seen between the treatments and the control groups. The control group's germination rate of 86.33% and the T4 group's germination rate of 64.00% ($p = 0.001$) were significantly different from one another when tested, with $p < 0.05$. The results for T2, T9, and T10, as well as the range of 65–67%, were the ones that came the closest. On the other hand, the weed species T1, T3, T5, T6, T7, T8, T11, and T13 had greater germination rates, ranging from 68.33 to 76.67% (Table 3).

Table 2. Physicochemical properties of soil used for pot experiments.

Properties	Value
Texture	Sandy loam
Sand (%)	58
Silt (%)	25
Clay (%)	17
pH	5.4
Organic carbon (%)	0.77
Organic matter (%)	1.33
Cation Exchange Capacity (CEC) (C.mol Kg^{-1})	6.48
Electrical conductivity (µS cm^{-1})	32.4
Total nitrogen (%)	0.11
Total phosphorus (%)	0.04
Total potassium (%)	0.34
Iron (%)	0.63
Manganese (mg Kg^{-1})	187.58
Zinc (mg Kg^{-1})	64.32
Available Bray-1 P (mg Kg^{-1})	9.0
Available K (meq/100 g)	0.19
Available Ca (meq/100 g)	1.89
Available Mg (meq/100 g)	1.03

Table 3. Effect of aqueous root exudates of weed species on the germination of *Triticum aestivum*.

Treatments (Weed Species)	Code	Petri Dish Experiments		Pot Experiments	
		Germination (%)	Mean Germination Time (MGT)	Germination (%)	Mean Germination Time (MGT)
Distilled water	C	86.33	4.44	87.23	3.54
Centella asiatica	T1	69.00 *	6.83 *	79.00	7.00 **
Rotala indica	T2	66.67 **	7.00 **	68.50 **	7.00 **
Solanum nigrum	T3	71.00 *	6.66 *	68.45 **	6.00
Commelina benghalensis	T4	64.00 ***	6.00	74.00 *	5.34
Marsilea quadrifolia	T5	68.33 **	7.00 **	70.00 **	6.00
Ageratum conyzoides	T6	70.00 **	8.00 **	69.33 **	6.00
Cynodon dactylon	T7	70.00 **	6.00	74.00 *	7.00 **
Spilanthes acmella	T8	76.67	6.56 *	73.50 *	6.33
Heliotropium indicum	T9	67.33 **	6.50 *	69.33 **	6.00
Leucas aspera	T10	67.67 **	6.17	68.67 **	6.50 *
Phyllanthus niruri	T11	71.67 ^{ns}	6.35	76.33	7.00 **
Sida acuta	T12	68. 67 **	6.32	69.33 **	6.33
Mikania micrantha	T13	73.33	6.81 *	84.33	5.33
Polygonum hydropiper	T14	65.33 **	7.00 **	70.00 **	6.50
Physalis heterophylla	T15	66.67 **	6.36 ^{ns}	69.33 **	6.68 *

* = $p < 0.05$, ** = $p < 0.001$, and *** = $p < 0.0001$; ns: not significant

In the pot experiment with root exudates, distilled water had the highest germination rate (87.23%). The same table showed that, as compared to pot culture, the germination percentage of *T. aestivum* seed in Petri dish culture, which was similarly impacted by root exudates, dramatically decreased. For the majority of treated weed root exudates, the germination rate varied between 74.00% to 68.50%, with the exception of T1, T3, T11, and T13, where the minimal germination rate was observed at 68.45% ($p = 0.001$).

3.2. Mean Germination Time (MGT)

There was a significant difference in the mean germination time (MGT) between the control and treatment groups. The T6 obtained the highest MGT of 8.00 ($p = 0.0025$) in the Petri dish, whereas the control received the lowest of 4.44. The following stance was supported by the T2, T5, and T14 of 7.00. Most of the treated rood exudates had mean germination times ranging from 6.01 to 6.83. In the case of the pot experiments, the T1, T2, T6, T7, and T11 obtained the highest MGT position in the pot of 7.00 ($p = 0.0010$), whereas the control received the lowest of 3.54. Most of the treated root exudates had mean germination times ranging from 6.01 to 6.83. T13, on the other hand, had the lowest MGT at 5.33. There were a few changes in the mean germination time of wheat in other plants treated with root exudates, ranging from 6.68 to 5.34 (Table 3).

3.3. Shoot Length

Root exudate in pots had a comparable influence on the shoot elongation of *T. aestivum.* The wheat's shoot length decreased as a result of the allelopathic action of weed types. The shortest shoot length (9.72 cm, $p = 0.0002$) was seen in wheat that received the root aqueous extract of the weed plant T13, while the longest shoot length (20.13 cm) was noticed when applied with the distilled water. The next lowest was in T11 (9.82 cm), which was followed by T10 (10.02 cm, $p = 0.0017$), T3 (10.33 cm, $p = 0.0047$), T8 (10.36 cm, $p = 0.0047$), and then T2 (10.43 cm, $p = 0.0011$). T12, T7, T4, T1, T6, and T15 weed's root extractions display an increased amount of shoot elongation at 10.58 cm, 10.83 cm, 10.87 cm, 11.72 cm, 11.98 cm, and 14.02 cm, respectively, in wheat. Among all the parameters, shoot length was non-significant in T14 (14.53 cm) and T5 (14.80 cm).

With regard to the Petri dish experiment, the ANOVA showed a significant inhibitory effect of the weed's root exudates upon the shoot length of the wheat. The maximum shoot growth (19.13 cm) was found in distilled water (control), which gradually reduced in T5

(18.04 cm), T9 (18.02 cm), T14 (17.39 cm), T15 (17.03 cm), and T6 (17.02 cm). It was shown that the highest reduction in shoot length was observed in T13 (12.01 cm, $p = 0.0074$). On the other hand, an amount of reduction (14.00–12.08 cm) was observed for the nine other varieties (Table 4).

Table 4. Effect of aqueous root exudates of weed species on the length of shoot and roots on *T. aestivum* seedlings.

Weed Species	Code	Petri Dish Experiments		Pot Experiments	
		Shoot Length (cm)	Root Length (cm)	Shoot Length (cm)	Root Length (cm)
Distilled water (Control)	C	19.13	3.46	20.13	6.42
Centella asiatica	T1	13.68 *	3.42	11.72 *	5.55 **
Rotala indica	T2	14.00	2.33 *	10.43 **	5.68 *
Solanum nigrum	T3	13.70 *	3.26	10.33 **	5.86
Commelina benghalensis	T4	12.63 **	2.31 *	10.87 **	5.32 **
Marsilea quadrifolia	T5	18.04	3.36	14.80	5.92
Ageratum conyzoides	T6	17.02	3.32	11.98 *	5.58 **
Cynodon dactylon	T7	12.70 **	2.19 **	10.83 **	5.62 *
Spilanthes acmella	T8	12.68 **	2.35 *	10.36 **	4.34 **
Heliotropium indicum	T9	18.02	3.22	13.67 *	4.92 **
Leucas aspera	T10	13.02 *	2.17 **	10.02 **	5.92
Phyllanthus niruri	T11	12.08 **	3.34	9.82 **	5.50 **
Sida acuta	T12	13.02 *	2.40 *	10.58 **	5.40 **
Mikania micrantha	T13	12.01 **	2.30 *	9.72 ***	4.64 **
Polygonum hydropiper	T14	17.39	3.24	14.53	5.38 **
Physalis heterophylla	T15	17.03	3.34	14.02 *	5.51 **

* = $p < 0.05$, ** = $p < 0.001$, *** = $p < 0.0001$; ns: not significant.

3.4. Root Length

In root exudate in the pot, the root lengths were between 6.42–4.34 cm. Like other seedling traits of the test plant, the control received the top position in root length (6.42 cm). Among all the weed species, T8 took the lowest position (4.34 cm, $p = 0.0042$), and the treatments T13 (4.64 cm) and T9 (4.92 cm) were close to the smallest possessor. The range of root length between 5.32–5.55 cm was demonstrated in T4 (5.32 cm), T14 (5.38 cm), T12 (5.40 cm), T11 (5.50 cm), T15 (5.51 cm), and T1 (5.55 cm). On the other hand, decreased root lengths were observed for the other 6 varieties. Among all the parameters, root length was non-significant in T3, T5, and T10 (Table 4).

The allelopathic impact often causes a process known as interruption of root elongation, which was also observed in the Petri dish because of the presence of aqueous weed root exudates. Based on root length, the control showed the maximum root length (3.46 cm), and T1 (3.42 cm) was close to the control. However, T7 and T10 presented the minimum root length (2.17 cm, $p = 0.0044$) compared to the other varieties. (3.36–2.30 cm), the range was observed in the other 12 varieties (Table 4).

3.5. Seedling Dry Weight

The significant allelopathic effect was also found in the seedling's dry weight in the aqueous root exudate of the weed species in the pot. The test plant, when treated with T8, contributed the minimum amount of dry matter production (19.71 mg, $p = 0.0025$), followed by T4 (20.78 mg), T14 (20.78 mg), and T5 (21.78 mg), respectively. The dry matter production range of the test plant was between 22.44 and 29.78 mg in 11 varieties. Among them, T2 showed the maximum amount of dry matter production (29.78 mg), which is near that of the control (33.33 mg) (Table 5).

Table 5. Estimation of dry biomass of *T. aestivum* seedlings that treated with aqueous root exudates of weed species.

Treatments (Weed Species)	(Code)	Petri Dish Experiments	Pot Experiments
		Seedling Dry Wt (mg Plant^{-1})	Seedling Dry Wt (mg Plant^{-1})
Distilled water (Control)	C	30.23	33.33
Centella asiatica	T1	27.32 *	27.00
Rotala indica	T2	28.44	29.78
Solanum nigrum	T3	19.55	25.89
Commelina benghalensis	T4	20.38 **	20.78 **
Marsilea quadrifolia	T5	22.34 **	21.78 **
Ageratum conyzoides	T6	25.33 **	25.33 *
Cynodon dactylon	T7	18.52 **	27.78
Spilanthes acmella	T8	23.43 **	19.71 **
Heliotropium indicum	T9	27.67 **	22.11 **
Leucas aspera	T10	23.51	26.89
Phyllanthus niruri	T11	27.33 **	23.78 **
Sida acuta	T12	27.67	26.56
Mikania micrantha	T13	24.33 **	25.89
Polygonum hydropiper	T14	19.00 **	20.78 **
Physalis heterophylla	T15	17.49 **	22.44 **

* = $p < 0.05$, ** = $p < 0.001$.

Root exudates created allelochemicals in the Petri dish, and these compounds impacted the formation of dry mass in the tested plant species. The greatest dry-matter production was in the control (30.23 mg), and the lowest was in T15 (17.49 mg, $p = 0.0024$). However, out of 15 weed species, T2 (28.44 mg) demonstrated the maximum amount of dry matter production. Overall, 27.67–22.34 mg of dry-matter reductions were observed in ten treatments (T1, T2, T5, T6, T8, T9, T10, T11, T12, and T13), and the other five treatments (T3, T4, T7, T14, and T15) scored 20.38–17.49 mg (Table 5).

3.6. Qualitative Phytochemicals Assay

According to the results in Table 6, all of the root exudates tested positive for alkaloids using various reagents such as Dragendroff's, Wagner's, Mayer's, Tannic acid, Hager's, and $FeCl_3$ (ferric chloride). In addition to alkaloids, qualitative assessments of carbohydrates, flavonoids, phenolics, and proteins were also performed. Carbohydrates were found in all exudates. All the tested species had low concentrations of flavonoids and phenolics, whereas proteins were not identified.

Table 6. Qualitative test for phytochemical screening of root exudates of fifteen weed species.

Weed Species	(Code)	Alkaloids						C.	Fla.	Phe.	P.
		D	W	M	T	H	Fe				
Centella asiatica.	T1	+	+++	+	+++	+++	+	+++	„	„	-
Rotala indica	T2	++	++	++	+++	+++	++	+++	„	„	-
Solanum nigrum	T3	+	++	++	+++	++	+	+	„	„	-
Commelina benghalensis	T4	+	+	+	++	+	+	++	„	„	-
Marsilea quadrifolia	T5	+	+	+	++	+	+	+	„	„	
Ageratum conyzoides	T6	+	+	+	+++	++	+	++	„	„	-
Cynodon dactylon	T7	++	++	+++	+++	++	++	+++	„	„	-
Spilanthes acmella	T8	+++	++	++	+++	+++	++	++	„	„	-
Heliotropium indicum	T9	+++	++	++	+++	+++	++	+++	„	„	
Leucas aspera	T10	++	++	++	+++	+++	+	+	„	„	-
Phyllanthus niruri	T11	+	+	+	+++	+	+	+++	„	„	-
Sida acuta	T12	+++	+++	+++	++	+++	++	++	„	„	-
Mikania micrantha	T13	+++	+++	+++	++		++	+++	„	„	-
Polygonum hydropiper	T14	++	++	++	+++	+++	++	++	„	„	-
Physalis heterophylla	T15	++	+	++	+++	++	+	+++	„	„	-

-: Not detected, „ : detected, + : Low concentration, ++ : Moderate concentration, +++: High concentration). D: Dragendroff's, W: Wagner's, M: Mayer's, T: Tannic acid, H: Hager's, Fe: $FeCl_3$ (Ferric chloride), C: Carbohydrate, Fla: Flavonoids, Phe: Phenolics, and P: Proteins.

3.7. Chlorophyll Content Assay

There were significant discrepancies in the data of the *T. aestivum* tested throughout the seedling growth stage (Figure 1). The seedlings were treated with 15 weed root exudates at 1 mL and 5 mL concentrations for Petri dishes and pot cultures, respectively. The first variation in content was in chlorophyll, where the level of chlorophyll a and b was decreased in both the Petri dish and the pot conditions compared to the control (Figures 2 and 3). However, in the Petri dish condition, both chlorophyll a and b declined more than in the pot condition on the 10th day. In addition, compared to the control, the inhibitory effect of T9, T11, and T12 on chlorophyll a and b was significantly lower ($p < 0.0001$) during seedling development than other root exudates in the Petri dish conditions.

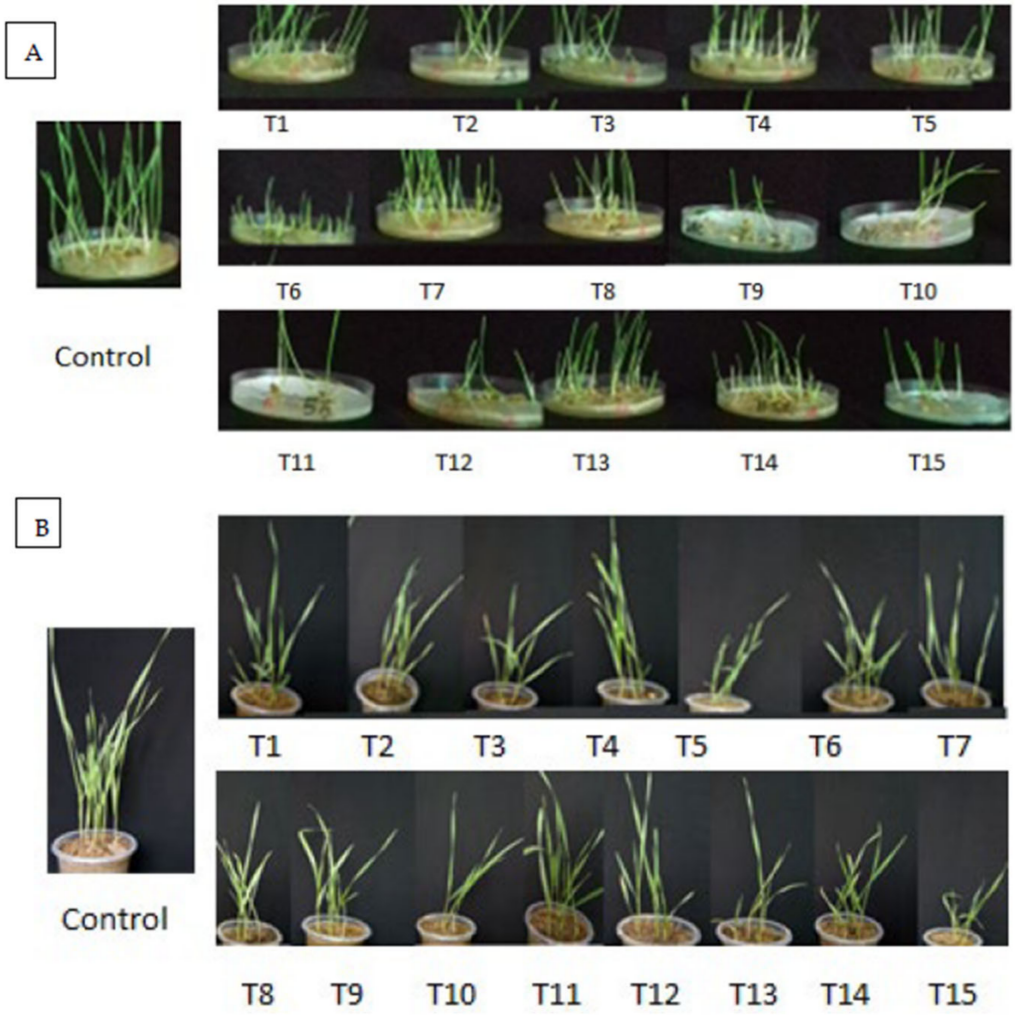


Figure 1. The images express the seedling emergences of wheat (*T. aestivum*) after 10 days in Petri dishes (**A**) and pot (**B**) cultivation that were treated with different root exudates of weeds. T1: *Centella asiatica*, T2: *Rotala indica*, T3: *Solanum nigrum*, T4: *ommelina benghalensis*, T5: *Marsilea quadrifolia*, T6: *Ageratum conyzoides*, T7: *Cynodon dactylon*, T8: *Spilanthes acmella*, T9: *Heliotropium indicum*, T10: *Leucas aspera*, T11: *Phyllanthus niruri*, T12: *Sida acuta*, T13: *Mikania micrantha*, T14: *Polygonum hydropiper*, T15: *Physalis heterophylla*.

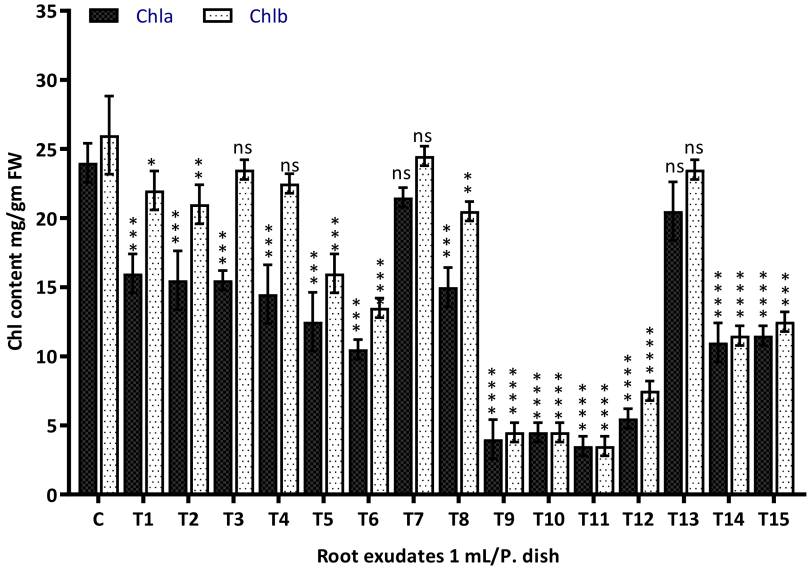


Figure 2. Effects of root exudates on chlorophyll a and chlorophyll b of *T. aestivum* shoot in Petri dishes cultivation. Different numbers of signs (*) indicate significant differences among the different root exudates of the tested weeds at the $p < 0.05$ level. ns = not significant. C = Control, T1: *Centella asiatica*, T2: *Rotala indica*, T3: *Solanum nigrum*, T4: *ommelina benghalensis*, T5: *Marsilea quadrifolia*, T6: *Ageratum conyzoides*, T7: *Cynodon dactylon,* T8: *Spilanthes acmella,* T9: *Heliotropium indicum,* T10: *Leucas aspera*, T11: *Phyllanthus niruri*, T12: *Sida acuta*, T13: *Mikania micrantha*, T14: *Polygonum hydropiper*, T15: *Physalis heterophylla*.

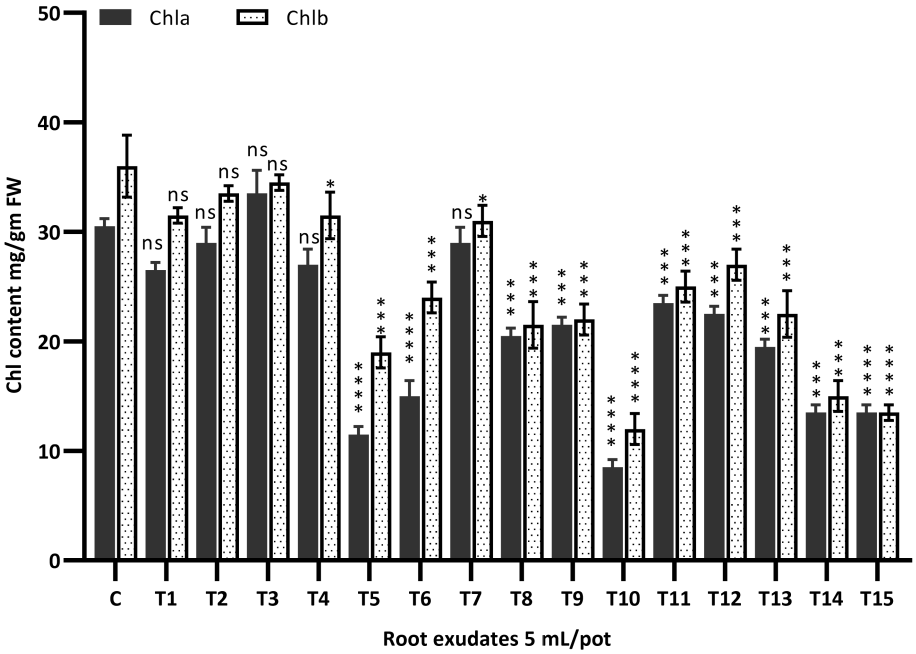


Figure 3. Effects of root exudates on chlorophyll a and chlorophyll b of *T. aestivum* shoot in pot cultivation. Different numbers of signs (*) indicate significant differences among the different root exudates of the tested weeds at the $p < 0.05$ level. ns = not significant. C = Control, T1: *Centella asiatica*, T2: *Rotala indica*, T3: *Solanum nigrum*, T4: *ommelina benghalensis*, T5: *Marsilea quadrifolia*, T6: *Ageratum conyzoides*, T7: *Cynodon dactylon,* T8: *Spilanthes acmella,* T9: *Heliotropium indicum,* T10: *Leucas aspera*, T11: *Phyllanthus niruri*, T12: *Sida acuta*, T13: *Mikania micrantha*, T14: *Polygonum hydropiper*, T15: *Physalis heterophylla*.

3.8. Antioxidant Enzymes Assay

Figures 4 and 5 show that seedlings' SOD and CAT activities were significantly ($p < 0.001$) lower in the root exudates of all weeds compared to the control. In most root exudates, the CAT activity of Petri dishes is greater than that of the pot condition. On the other hand, the SOD activity of the pot condition is exceptionally higher than that of the Petri dish condition in all root exudates. In contrast to the preceding parameter, the MDA content of *T. aestivum* seedlings increased considerably for most of the root exudates ($p < 0.001$) when compared to the control groups. Furthermore, MDA levels were higher in the Petri dish condition than in the pot cultivation in all cases when compared to the control (Figure 6).

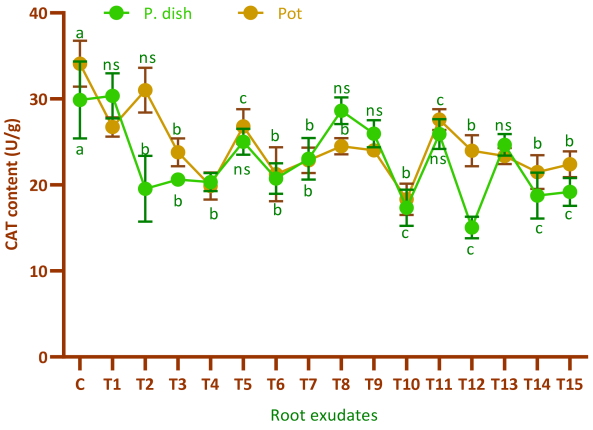


Figure 4. Catalase (CAT) of 10 days old seedlings of *T. astivum* in Petri dish and pot condition after irrigated with 15 weeds root exudates 1 mL and 5 mL, respectively. Different letters (a, b, and c) indicate significant differences with control at $p < 0.05$ level. ns = not significant. C = Control, T1: *Centella asiatica*, T2: *Rotala indica*, T3: *Solanum nigrum*, T4: *ommelina benghalensis*, T5: *Marsilea quadrifolia*, T6: *Ageratum conyzoides*, T7: *Cynodon dactylon*, T8: *Spilanthes acmella*, T9: *Heliotropium indicum*, T10: *Leucas aspera*, T11: *Phyllanthus niruri*, T12: *Sida acuta*, T13: *Mikania micrantha*, T14: *Polygonum hydropiper*, T15: *Physalis heterophylla*.

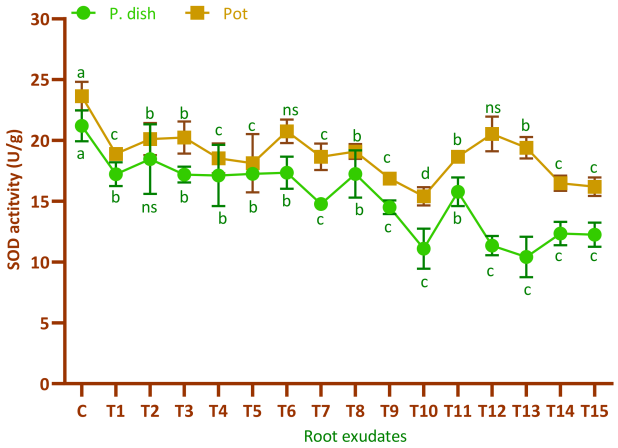


Figure 5. Superoxide dismutase (SOD) contents of 10 days old seedlings of *T. astivum* in Petri dishes and pot condition after irrigated with 15 weeds root exudates 1 mL and 5 mL, respectively. Different letters (a, b, c, and d) indicate significant differences with control at $p < 0.05$ levels. ns = not significant. C = Control, T1: *Centella asiatica*, T2: *Rotala indica*, T3: *Solanum nigrum*, T4: *ommelina benghalensis*, T5: *Marsilea quadrifolia*, T6: *Ageratum conyzoides*, T7: *Cynodon dactylon*, T8: *Spilanthes acmella*, T9: *Heliotropium indicum*, T10: *Leucas aspera*, T11: *Phyllanthus niruri*, T12: *Sida acuta*, T13: *Mikania micrantha*, T14: *Polygonum hydropiper*, T15: *Physalis heterophylla*.

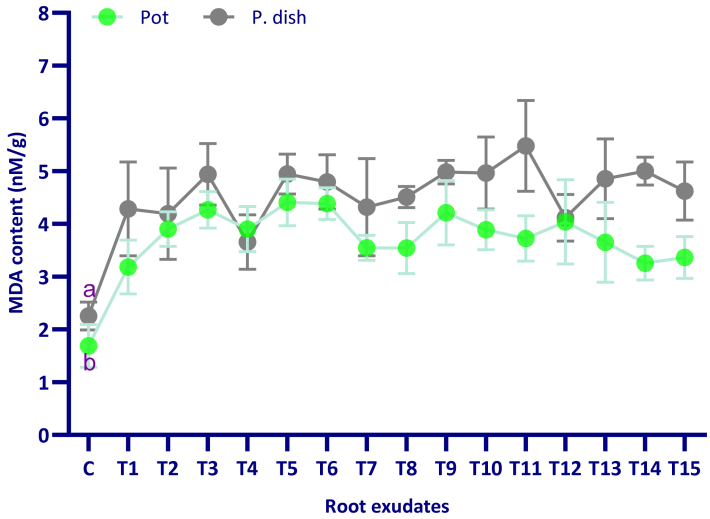


Figure 6. Malondialdehyde (MDA) contents of 10 days old seedlings of *T. astivum* in Petri dishes and pot conditions after irrigation with 15 weeds root exudates of 1 mL and 5 mL, respectively. The letters "a" and "b" indicate the significant differences between Petri dishes and pot culture, respectively, at $p < 0.05$ levels with control. ns = not significant. C = Control, T1: *Centella asiatica*, T2: *Rotala indica*, T3: *Solanum nigrum*, T4: *ommelina benghalensis*, T5: *Marsilea quadrifolia*, T6: *Ageratum conyzoides*, T7: *Cynodon dactylon*, T8: *Spilanthes acmella*, T9: *Heliotropium indicum*, T10: *Leucas aspera*, T11: *Phyllanthus niruri*, T12: *Sida acuta*, T13: *Mikania micrantha*, T14: *Polygonum hydropiper*, T15: *Physalis heterophylla*.

4. Discussion

Root exudates play an important role in plant–environment interactions. The diverse allelochemicals need varied procedures for collecting root exudates, with most being extracted using either water or organic solvent [28]. Tolerance of environmental stress and change, as well as fast growth due to the production of huge amounts of biomass in a short amount of time, are two defining characteristics of weed plants. Plants are negatively impacted by weeds due to allelopathic effects and direct competition for resources [29]. The results of this study showed that root exudates of 15 field weeds have allelopathic effects on the germination of seeds. The weeds T8 in a pot and T13 in a Petri dish show an increasing positive effect on the seed germination of wheat as compared to the others, indicating a positive allelopathic effect of these weeds on the wheat. The weeds T2, T4, T5, and T9 showed a slightly low germination rate in the Petri dish; on the other hand, the same weeds showed a slightly higher germination rate in the pot experiment [30]. All of the weeds studied showed that concentrated root exudate inhibited seed development. Weed toxins that seep into the soil may have an allelopathic impact on subsequent crop plants. Some weed species' dominance in a field may be attributable to their ability to inhibit the growth of other weeds via allelopathic activity. Allelopathy is caused by the production of phytotoxic compounds from plant residues, which can result in seed germination delays or inhibition, as well as poor crop stand. Several other studies have shown similar outcomes, reinforcing these conclusions [29,31,32].

Germination percentages, rates (speeds), and uniformity can vary between seeds from various individuals, populations, seed lots, and treatments. The "mean time to germination", rates (speeds), and uniformity can vary between seeds from various individuals, populations, seed lots, and treatments. The "mean time to germination", a measurement of the rate and time-spread of germination, has a drawback when used to determine the germination rate. Mean germination time (MGT) does not display the amount of time from the beginning of imbibition to a special germination rate. MGT has been used to compare particular pairs or groups of means and gauge seed vigor. Thus, this study calculated the MGTs in differences between the germination of seeds in a Petri dish and in a pot with

soil [33]. The interactive effect of all tested weed root exudates resulted in a significant effect on the MGT value of wheat seeds. Similarly, the MGT values of the control were lower than those of the Petri dish. Another study that supported our research revealed higher MGT in a non-soil germination test [34].

Plant shoot and root lengths are reliable indicators of weed–crop competition. In general, the crop's shoot and root lengths were significantly decreased as weed–crop competition increased. Even though weeds compete with the crop, weed allelopathy is the most harmful factor that slows crop development. In this work, we found that the shoot length of wheat seedlings was significantly affected by the interplay of root exudate from various weeds. Previous investigations also found that root exudate impeded root and shoot growth, suggesting that it included harmful compounds [35,36]. Our findings are consistent with those of previous research, which found that wheat seedlings were negatively affected by residues of allelopathic weeds, not owing to a lack of soil nutrients but rather because of the weeds' water-soluble phenolic acid release. Channappagoudar and Agasimani [37] stated that phenolic compounds are major phytotoxins which cause inhibition in the early seedling growth of plants.

The interaction between various exudates and tested species revealed that the control treatment produced the most significant levels of dry biomass, while treatments with T8 and T11 produced the lowest levels. As a result, *T. aestivum* exhibits varying tolerance, perhaps as a result of genetic predisposition. These findings are in line with the findings of Kapoor et al. [38], who claimed that aqueous weed exudate drastically decreased the biomass of field crops. More inhibitory compounds were found in residues when wheat seedlings' total dry weight was reduced to its lowest possible level. There is a positive correlation between allelochemical content and allelopathic effect on wheat germination and growth, as shown by Shahrokhi et al. [39].

Allelochemical stress affects the physiological indices of crop seedlings. We found that soil in both the pot and Petri dish significantly reduced the chlorophyll content of *T. aestivum* seedlings and that the impact of Petri dish cultivation was much more significant than that of the pot, which is consistent with the other findings [40]. Furthermore, the root exudates of some weeds such as T9, T10, T11, and T14 are more vigorous than the other weeds. On the other hand, T5, T6, T10, and T15 are more effective in terms of chlorophyll content. High concentration and extended exposure to allelochemicals impair chlorophyll synthesis, reduce stomatal opening, and reduce the stomatal opening ratio in seedlings of *T. aestivum* grown in a Petri dish compared to a pot culture [41].

Furthermore, allelopathic stress may increase plants' reactive oxygen species (ROS), producing oxidative stress [42]. Overproduction of ROS causes oxidative stress and may harm biological components. To combat the consequences of oxidative stress, plants have developed a complex system of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), as well as various non-enzymatic antioxidants. However, when the quantity of allelochemicals is high enough, the internal defense system of plants fails once a certain threshold is reached, and the antioxidant enzyme activity of plants decreases dramatically. Our study found that SOD and CAT activities in *T. aestivum* seedlings were significantly lower than those in the control group, consistent with other findings [41,43]. In addition, root exudates concentration at 5 mL moderately increased during the pot condition compared to in the Petri dishes condition, supported by Song et al. [44].

Allelochemicals may cause membrane lipid peroxidation by upsetting the equilibrium between free radical generations and the scavenging mechanism in plant tissue. MDA (malondialdehyde) is a byproduct of this process. The MDA level of *T. aestivum* seedlings was substantially higher after exposure to root exudates than in the control. Furthermore, MDA concentration exposed to Petri dishes was substantially more significant than that of the soil (pot) condition, especially the root exudate of T1, T3, T5, T6, T9, T10, and T11. So, we concluded that both pots and Petri dishes could increase the antioxidant enzyme activity of *T. aestivum* seedlings, speed up the buildup of reactive oxygen species in cells, and eventually cause damage to the structure and function of membranes, making them

more permeable. It is also remarkable that the soil condition of seedlings affects the Petri dishes condition less for antioxidant enzymes; this may be due to the characteristics of soil components [41].

5. Conclusions

The root exudates of fifteen common invasive weeds on the Chittagong University campus have a negative effect on the growth of *T. aestivum* seeds and seedlings. The allelopathic effect of *Rotala indica. Commelina benghalensis, Heliotropium indicum, Leucas aspera, Polygonum hydropiper,* and *Physalis heterophylla* at the same concentrations was more substantial than that of the other root exudates of weeds in Petri dishes and pot cultivations. In contrast, the soil in the pot cultivations was relatively less effective than the Petri dishes in seed germination and seedling growth. Furthermore, the same exudates might cause a relative reduction in the activity of the antioxidant enzymes CAT and SOD, as well as an increase in MDA content, influencing the germination rate of *T. aestivum* seeds and the development of seedling roots and shoots. Finally, in all situations, weed root exudates in Petri dishes cultivation show a more significant detrimental impact than those in pot culture.

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References

1. Scavo, A.; Mauromicale, G. Crop allelopathy for sustainable weed management in agroecosystems: Knowing the present with a view to the future. *Agronomy* **2021**, *11*, 2104. [CrossRef]
2. Farooq, M.; Jabran, K.; Cheema, Z.A.; Wahid, A.; Siddique, K.H.M. Allelopathy for weed management. In *Co-Evolution of Secondary Metabolites*; Springer: Berlin/Heidelberg, Germany, 2020; pp. 505–519.
3. Kato-Noguchi, H.; Kurniadie, D. Allelopathy and allelochemicals of Leucaena leucocephala as an invasive plant species. *Plants* **2022**, *11*, 1672. [CrossRef] [PubMed]
4. Tesio, F.; Ferrero, A. Allelopathy, a chance for sustainable weed management. *Int. J. Sustain. Dev. World Ecol.* **2010**, *17*, 377–389. [CrossRef]
5. Sangi, D.P.; Meira, Y.G.; Moreira, N.M.; Lopes, T.A.; Leite, M.P.; Pereira-Flores, M.E.; Alvarenga, E.S. Benzoxazoles as novel herbicidal agents. *Pest Manag. Sci.* **2019**, *75*, 262–269. [CrossRef] [PubMed]
6. Jabran, K.; Farooq, M. Implications of potential allelopathic crops in agricultural systems. In *Allelopathy*; Springer: Berlin/Heidelberg, Germany, 2013; pp. 349–385.
7. Masum, S.M.; Hossain, M.A.; Akamine, H.; Sakagami, J.I.; Bhowmik, P.C. Allelopathic potential of indigenous Bangladeshi rice varieties. *Weed Biol. Manag.* **2016**, *16*, 119–131. [CrossRef]
8. Sangeetha, C.; Baskar, P. Allelopathy in weed management: A critical review. *Afr. J. Agric. Res.* **2015**, *10*, 1004–1015.
9. Masum, S.M.; Hossain, M.A.; Akamine, H.; Sakagami, J.I.; Bhowmik, P.C. Allelopathic effects of sesame extracts on seed germination of moso bamboo and identification of potential allelochemicals. *Sci. Rep.* **2022**, *12*, 6661.
10. Kato-Noguchi, H. Allelopathy and Allelochemicals of Imperata cylindrica as an invasive plant species. *Plants* **2022**, *11*, 2551. [CrossRef]
11. Qasem, J.R. Allelopathy Field Research Methods. 1. Crops-weeds interactions. *Allelopath. J.* **2012**, *30*, 159–176.

12. Lopes, R.W.N.; Marques Morais, E.; Lacerda, J.J.D.J.; Araújo, F.D.D.S. Bioherbicidal potential of plant species with allelopathic effects on the weed *Bidens bipinnata* L. *Sci. Rep.* **2022**, *12*, 13476. [CrossRef]
13. Ishak, M.; Iberahim, Z.; Mardiana-Jansar, K. Comparative response of Malaysian weedy rice (*Oryza sativa*) initial growth towards the allelopathic potential of *Leucaena leucocephala* (Lam.) de Wit and Dicranopteris linearis (Burm. f.) Underw. In *IOP Conference Series: Earth and Environmental Science*; IOP Publishing: Bristol, UK, 2021.
14. Chon, S.-U.; Nelson, C. Allelopathy in Compositae plants. A review. *Agron. Sustain. Dev.* **2010**, *30*, 349–358. [CrossRef]
15. Tudi, M.; Ruan, H.D.; Wang, L. Agriculture development, pesticide application and its impact on the environment. *Int. J. Environ. Res. Public Health* **2021**, *18*, 1112. [CrossRef] [PubMed]
16. Hoang Anh, L.; Van Quan, N.; Tuan Nghia, L.; Dang Xuan, T. Phenolic allelochemicals: Achievements, limitations, and prospective approaches in weed management. *Weed Biol. Manag.* **2021**, *21*, 37–67. [CrossRef]
17. Cheng, F.; Cheng, Z. Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. *Front. Plant Sci.* **2015**, *6*, 1020. [CrossRef]
18. Alsharekh, A.; El-Sheikh, M.A.; Alatar, A.A.; Abdel-Salam, E.M. Natural control of weed invasions in hyper-arid arable farms: Allelopathic potential effect of Conocarpus erectus against common weeds and vegetables. *Agronomy* **2022**, *12*, 703. [CrossRef]
19. Cromwell, B. The alkaloids. In *Modern Methods of Plant Analysis/Moderne Methoden der Pflanzenanalyse*; Springer: Berlin/Heidelberg, Germany, 1955; pp. 367–516.
20. Boxi, M.; Rajesh, Y.; Kumar, V.R.; Praveen, B.; Mangamma, K. Extraction, phytochemical screening and in-vitro evaluation of anti-oxidant properties of Commicarpuschinesis (aqueous leaf extract). *Int. J. Pharm. Biosci.* **2010**, *1*, 537–547.
21. Sofowora, A. Recent trends in research into African medicinal plants. *J. Ethnopharmacol.* **1993**, *38*, 197–208. [CrossRef] [PubMed]
22. Hiscox, J.; Israelstam, G. A method for the extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot.* **1979**, *57*, 1332–1334. [CrossRef]
23. Arnon, D.I. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* **1949**, *24*, 1. [CrossRef]
24. Beers, R.F.; Sizer, I.W. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J. Biol. Chem.* **1952**, *195*, 133–140. [CrossRef]
25. Kakkar, P.; Das, B.; Viswanathan, P. A modified spectrophotometric assay of superoxide dismutase. *Indian J. Biochem. Biophys.* **1984**, *21*, 130–132. [PubMed]
26. Fridovich, I. Fundamental aspects of reactive oxygen species, or what's the matter with oxygen? *Ann. N. Y. Acad. Sci.* **1999**, *893*, 13–18. [CrossRef] [PubMed]
27. Högberg, J.; Larson, R.E.; Kristoferson, A.; Orrenius, S. NADPH-dependent reductase solubilized from microsomes by peroxidation and its activity. *Biochem. Biophys. Res. Commun.* **1974**, *56*, 836–842. [CrossRef]
28. Wang, C.; Qi, J.; Liu, Q.; Wang, Y.; Wang, H. Allelopathic Potential of Aqueous Extracts from Fleagrass (Adenosma buchneroides Bonati) against Two Crop and Three Weed Species. *Agriculture* **2022**, *12*, 1103. [CrossRef]
29. Qasem, J. A Survey on the phytotoxicity of common weeds, wild grown species and medicinal plants on wheat. *Allelopath. J.* **2017**, *42*, 179–194. [CrossRef]
30. Ramadan, T.; Amro, A.; Alazazi, S. Comparative allelopathic potential of ten field weeds against seed germination of three economic plants. *Biol. Forum. –Int. J.* **2018**, *10*, 168–181.
31. Bachheti, A.; Sharma, A.; Bachheti, R.K.; Husen, A.; Pandey, D.P. Plant allelochemicals and their various applications. In *Co-Evolution of Secondary Metabolites*; Springer: Berlin/Heidelberg, Germany, 2020; pp. 441–465.
32. Shi, S.; Cheng, J.; Ahmad, N.; Zhao, W.; Tian, M.; Yuan, Z.; Zhao, C. Effects of potential allelochemicals in a water extract of Abutilon theophrasti Medik. on germination and growth of *Glycine max* L., *Triticum aestivum* L., and *Zea mays* L. *J. Sci. Food Agric.* 2022, *ahead of print*. [CrossRef]
33. Soltani, E.; Ghaderi-Far, F.; Baskin, C. Problems with using mean germination time to calculate rate of seed germination. *Aust. J. Bot.* **2015**, *63*, 631–635. [CrossRef]
34. Benvenuti, S. Soil texture involvement in germination and emergence of buried weed seeds. *Agron. J.* **2003**, *95*, 191–198. [CrossRef]
35. Zareen, S.; Fawad, M.; Haroon, M.; Ahmad, I.; Zaman, A. Allelopathic potential of summer weeds on germination potential and growth performance of wheat and chickpea. *J. Nat. Pestic. Res.* **2022**, *1*, 100002. [CrossRef]
36. Moreno-Robles, A.; Cala Peralta, A.; Soriano, G.; Zorrilla, J.G.; Masi, M.; Vilariño-Rodríguez, S.; Fernández-Aparicio, M. Identification of Allelochemicals with Differential Modes of Phytotoxicity against Cuscuta campestris. *Agriculture* **2022**, *12*, 1746. [CrossRef]
37. Channappagoudar, B.; Agasimani, C. Physiological studies on weed control effiCiency in garlic. In Proceedings of the National Seminar on New Perspectives in Spices, Medicinal and Aromatic Plants, 27–29 November 2003; Indian Society for Spices: Kozhikode, Kerala, India, 2003; p. 169.
38. Kapoor, R.T.; Srivastava, A.K.; Meenal, A. Allelotoxicity of aqueous leaf extracts of *Parthenium hysterophorus* L. on the biomass and physiological-behaviour of *Brassica juncea* and *Zea mays*. *Electron. J. Environ. Agric. Food Chem.* **2012**, *11*, 53–67.
39. Shahrokhi, S.; Darvishzadeh, M.; Mehrpouyan, M.; Farboodi, M.; Akbarzadeh, M. Germination and growth of wheat, Triticum aestivum (cv. azar2) in response to Pigweed, *Amaranthus retroflexus* L. organs extracts. In Proceedings of the 2nd International Conference on Agricultural and Animal Science, Maldives, 5–27 November 2011.

40. Kim, L.R.; Adhikari, A.; Kang, Y.; Gam, H.J.; Kang, S.M.; Kim, K.Y.; Lee, I.J. Investigation of Solanum carolinense Dominance and Phytotoxic Effect in Festuca arundinacea with Special Reference to Allelochemical Identification, Analysis of Phytohormones and Antioxidant Mechanisms. *Agronomy* **2022**, *12*, 1954. [CrossRef]
41. Ma, H.; Chen, Y.; Chen, J.; Zhang, Y.; Zhang, T.; He, H. Comparison of allelopathic effects of two typical invasive plants: Mikania micrantha and Ipomoea cairica in Hainan island. *Sci. Rep.* **2020**, *10*, 11332. [CrossRef]
42. Inderjit. Plant phenolics in allelopathy. *Bot. Rev.* **1996**, *62*, 186–202.
43. Talukder, M.R.; Asaduzzaman, M.; Ueno, M.; Tanaka, H.; Asao, T. Alleviation of allelochemical stress-induced growth inhibition and oxidative damage in lettuce under closed hydroponics through electro-degradation. *Hortic. Sci.* **2020**, *47*, 53–68. [CrossRef]
44. Song, L.; Pan, K.W.; Wang, J.C.; Ma, Y.H. Effects of phenolic acids on seed germination and seedling antioxidant enzyme activity of alfalfa. *Acta Ecol. Sin.* **2006**, *26*, 3393–3403.

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